AD_____

GRANT NUMBER: DAMD17-94-J-4231

TITLE: Estrogen Metabolism and Familial Risk of Breast Cancer

PRINCIPAL INVESTIGATOR: Giske Ursin, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of Southern California
School of Medicine
Los Angeles, California 90033

REPORT DATE: October 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1995	Annual 1 Oct 94 - 30 Sep 95

4. TITLE AND SUBTITLE
Estrogen Metabolism and Familial Risk of Breast Cancer

5. FUNDING NUMBERS
DAMD17-94-J-4231

6. AUTHOR(S)
Giske Ursin, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Southern California School of Medicine
Los Angeles, California 90033

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

It has been suggested that women who metabolize a larger proportion of their natural estrogen via the 16α-hydroxy pathway may be at significantly elevated risk of breast cancer compared to women who metabolize proportionally more estrogen via the 2-hydroxy pathway. This study evaluates whether the ratios of 16α–OHE1 to 2-OHE1 are higher in urine of premenopausal women at "high" than at "low" familiar risk of breast cancer; and whether the ratio is elevated in cases independent of total urinary estrone (E1), estradiol (E2) and estriol (E3). Early morning urine samples are collected from 100 premenopausal women at "high" and 100 premenopausal women at "low" risk of breast cancer. All subjects are sisters or daughters of subjects participating in one of three case-control studies of breast cancer at our institution. Five estrogen metabolites in urine are determined: 16α–OHE1, OHE1, E1, E2 and E3 conjugates. The data collection is in progress.

14. SUBJECT TERMS	15. NUMBER OF PAGES
estrogen metabolism, 16α- and 2-hydroxyestrone, urine, familial risk of breast cancer, case-control study, breast cancer	8
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	**PR**	- Project
G	- Grant	**TA**	- Task
PE	- Program Element	**WU**	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

- **DOD** - See DoDD 5230.24, "Distribution Statements on Technical Documents."
- **DOE** - See authorities.
- **NASA** - See Handbook NHB 2200.2.
- **NTIS** - Leave blank.

Block 12b. Distribution Code.

- **DOD** - Leave blank.
- **DOE** - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
- **NASA** - Leave blank.
- **NTIS** - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

__✓__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

10/24/95

PI - Signature Date

(4) Table of Contents:

	Pages
(1) Front Cover	1
(2) SF 298 Report Documentation Page	2
(3) Foreword	3
(4) Table of Contents	4
(5) Introduction	5
(6) Body	5
(7) Conclusions	8
(8) References	8

(5) Introduction:

16α- and 2-hydroxylation of E1

The extent to which E1 is metabolized via the 16α-hydroxylation pathway may be associated with breast cancer risk (1-3). The two main pathways for metabolizing E1 are via 16α -hydroxylation and 2-hydroxylation . The 16α-metabolites, are biologically active; the 2-metabolites are not (4-6).

Women at high risk of breast cancer

Women who have a first degree relative who has had breast cancer are themselves at increased risk. The risk is higher if the relative had bilateral compared to unilateral breast cancer, or if the diagnosis was made at an early age. Women who had a first degree relative diagnosed with bilateral breast cancer before age 50 may have as much as a five-fold elevated risk (7-12)

The increased risk in first degree family members may be due to shared environmental factors and/or genetic factors that increase the susceptibility to breast cancer. One possible mechanism for the increased risk (or part of the increased risk) could be the pathway by which estrogen is metabolized (13).

The hypothesis to be tested is that women at 'high-risk' of breast cancer metabolize a significantly higher amount of E1 through 16α- than 2-hydroxylation compared to 'normal-risk' women, independent of total urinary E1, E2 and E3. We expect the ratios of 16α-hydroxy-metabolites to 2-hydroxy-metabolites to be statistically significantly higher in 'high-risk' than in 'normal-risk' women.

(6) Body

METHODS

Case selection: We are in the process of identifying 100 women at high risk of breast cancer. Premenopausal sisters and daughters of patients with premenopausal uni- or bilateral breast cancer who participated in a genetic-epidemiologic study (14) or a case-control study of breast cancer (15-16), and who have never themselves been diagnosed with breast cancer, represent the 'cases' in this study.

Case selection- eligibility criteria:

1. Premenopausal sister or daughter of a woman with either
   a) premenopausal bilateral breast cancer who was identified through the Los Angeles County Cancer Surveillance Program (LACCSP; and NCI SEER registry), and participated in a genetic-epidemiologic study of breast cancer (P.I. Robert W. Haile), or
   b) unilateral breast cancer diagnosed before the age of 40 who was identified through the LACCSP, and participated in a study of breast cancer (P.I. Leslie Bernstein)
2. Age between 20 and 50 years old.
3. Not currently pregnant or breast feeding.
4. Never been diagnosed with cancer.
5. Over the past 6 months: not used medications that may interfere with estrogen metabolism (estrogen, progesterone, oral contraceptives, tamoxifen, cimetidine, thyroxin, or omega-3 fatty acid supplements).
6. Over the past 3 months: not had general anesthesia.

7. Living in California.

Control selection- eligibility criteria

We originally proposed to use 100 age-matched female friends of the cases as controls. However, because of concerns regarding use of friends as controls, as well as substantial problems obtaining permission from the cases to contact their friends (less than 10% of cases were willing to provide us with names of friends), we had to find other controls. We are now using daughters or sisters of controls who participated in one of two studies of breast cancer conducted by Dr. Leslie Bernstein in our department.

Eligible controls are:

1. Premenopausal sister or daughter of a woman who participated in either
   a) study of breast cancer under the age of 40 (P.I. Leslie Bernstein) or
   b) the USC part of the Women's CARE study (USC P.I. Leslie Bernstein)
2. Age between 20 and 50 years old.
3. Not currently pregnant or breast feeding.
4. Never been diagnosed with cancer.
5. Over the past 6 months: not used medications that may interfere with estrogen metabolism (estrogen, progesterone, oral contraceptives, tamoxifen, cimetidine, thyroxin, or omega-3 fatty acid supplements).
6. Over the past 3 months: not had general anesthesia.
7. Living in California.

If more than one member is eligible in one family, then the youngest member is enrolled in our study.

Data acquisition

We contact women who have participated in one of our previous studies, and ask them for permission to contact any daughters or sisters they may have living in California, and who are between the ages of 20 and 50 years old. We contact these daughters/sisters, and find out if they are eligible for the study.

We obtain urine samples from the women during the follicular phase, i.e. during the first 10 days of the menstrual cycle. Once a woman is found to be eligible, she is asked to call us when the next menstrual cycle starts.

A box containing a 100 ml urine vial with a 100 mg ascorbate tablet, a small cooler with an ice pack, an informed consent form, and a questionnaire on recent intake of medication, alcohol and specific foods, are shipped to each eligible woman who agrees to participate. The participants are asked to place the urine sample in the cooler with the ice pack (previously frozen by the participant) immediately after it has been produced, and to enclose a signed informed consent form and the completed questionnaire on alcohol intake and current medication. The cooler is shipped to us by overnight express mail. The urine samples are divided into four samples of approximately 12 ml and immediately frozen at -70°C until shipped to the processing laboratories. Dr. Bradlow at the Strang-Cornell Cancer Research Laboratory will perform the 16α-OHE1 and 2-OHE1 assays while Dr. Stanczyk at Los Angeles County/University of Southern California (LAC/USC) Women's Hospital will perform the E1, E2 and E3 assays. The only identifiers on the samples are code numbers ensuring that the laboratories will be blinded as to case or control status of the individual samples. Dietary questionnaires are sent to each woman

one week after she provides the urine sample. The dietary questionnaire is returned by mail in a stamped envelope we provide.

RESULTS

We have so far contacted 461 women with premenopausal uni- or bilateral breast cancer and 413 controls who participated in one of our previous studies (see above). Initial attempts were made to contact these women by telephone. However, most of these women were last contacted as part of the previous study some time before 1990. Therefore, a large number of the telephone numbers were no longer current. We therefore early decided to make the initial contact by mail, with a request for a forwarding address. We have sent up to three letters to some of these women. We have attempted to track women who's current address is unknown (their letters have been returned with no forwarding address), through the records of the California Department of Motor Vehicles. In addition, we have attempted to call non-responders, using their old number, or any new number we have found using the nanes inverse street directory. Completing this search and obtaining responses from these women turned out to be a much greater task than expected (first part of task 1 in original Statement of Work (SOW)).

Of the responses obtained so far from the cases and controls in one of our previous studies, 181 cases and 124 controls had at least one daughter or sister between the ages of 20 and 50 living in California (total of 262 case daughters or sisters and 205 control daughters or sisters). We have contacted all of these case and control daughters and sisters. When there is more than one daughter/sister in each family, we include the youngest one above age 20 if eligible. This means that we must await a response from the younger potential eligible member before we decide who should be included in the study. To date we have obtained responses from approximately 180 case daughters or sisters and 140 control daughters or sisters. Since only one member from each family is eligible, this means that we have a total of 78 eligible case daughters or sisters and 28 eligible control daughters or sisters. Major reasons for ineligibility include current oral contraceptive use (30%), current smokers (10%), other medications (5-10%), irregular periods (10% of controls), currently pregnant/breast feeding (10% of controls). Finding eligible women (especially controls) therefore represented an enormous problem (task 1 in SOW). We have so far collected urine samples and dietary questionnaires on 70 case daughters or sisters and 20 control daughters or sisters (tasks 2a and 2c in SOW). We are contacting eligible women on a regular basis to remind them of contacting us when their menstrual period begins. We are now making a final effort to increase the number of control participants.

We have not started shipping urine samples to the laboratories (tasks 2b and 3 in SOW) for two reasons: 1) due to the substantial problems finding eligible controls, and 2) because our study coincided with a reproducibility/validity study of the EIA assays of 16α- and 2-OHE1 conducted by Dr. Regina Ziegler, NCI. The results of this validation study indicated that the assays must be adjusted (Regina Ziegler, personal communication), and as a result the new adjusted assays must again be validated. We therefore requested (and obtained) a 1-year no-cost extension of this grant.

All dietary questionnaires will be shipped to Harvard once the study is completed. The other risk factor information obtained for this study is being prepared for key-punching. The data will be key-punched all at one time when the data collection is completed.

(7) Conclusions

We have no laboratory results we can draw implications from at this point. It is also too early to suggest changes for future projects, except perhaps that it would be useful to request funds for a separate validity/reproducibility study whenever a new method is being used.

(8) References

1. Bradlow HL, Hershcopf RE, Fishman JF. Oestradiol 16alpha-hydroxylase:a risk marker for breast cancer. Cancer Surv 1986;5:574-83.
2. Bradlow HL, Hershcope R, Martucci C, Fishman J. 16α-hydroxylation of estradiol: A possible risk marker for breast cancer. Ann NY Acad Sci 1986;464:138-51.
3. Bradlow HL, Hershcopf RE, Martucci CP, Fishman J. Estradiol 16 alpha-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: A possible model for the hormonal etiology of breast cancer in humans. Proc Natl Acad Sci USA;1985:82:6295-9.
4. Clark JH, Paszko Z, Peck EJ Jr. Nuclear binding and retention of the receptor estrogen complex:relation to the agonistic and antagonistic properties of estriol. Endocrinol 1977;100:91-6.
5. Fishman J, Martucci C. Biological properties of 16alpha-hydroxyoestrone:implications in estrogen physiology and pathophysiology. J Clin Endocrin Metab 1980;51:611-5.
6. Martucci C, Fishman J. Direction of estradiol metabolism as a control of its hormonal action - uterotrophic activity of estradiol metabolites. Endocrinol 1977;101:1709-15.
7. Anderson DE. Some characteristics of familial breast cancer. Cancer 1971;28:1500-4.
8. Anderson DE. A genetic study of human breast cancer. J Nat Cancer Inst 1972;48:1029-34.
9. Bain C, Speizer FE, Rosner B, Belanger C, Hennekens CH. Family history of breast cancer as a risk indicator for the disease. Am J Epidemiol 1980;111:301-8.
10. Ottman R, Pike MC, King M-C, Henderson BE. Practical guide for estimating risk for familial breast cancer. Lancet 1983;ii:556-8.
11. Ottman R, Pike MC, King M-C, Casagrande JT, Henderson BE. Familial breast cancer in a population-based series. Am J Epidemiol 1986;123:15-21.
12. Colditz GA, Willett WC, Hunter DJ, Stampfer MJ, Manson JE, Hennekens CH, Rosner BA, Speizer FE. Family history, age, and risk of breast cancer. Prospective data from the Nurses' Health Study. J Am Med Assoc 1993;270:338-43.
13. Osborne MP, Karmali RA, Hershcopf RJ, Bradlow HL, Kourides IA, Williams WR, Rosen PP, Fishman J. Omega-3 fatty acids:modulation of estrogen metabolism and potential for breast cancer prevention. Cancer Invest 1988;8:629-31.
14. Ursin G, Aragaki CA, Paganini-Hill A, Siemiatycki J, Thompson WD, Haile RW. Oral contraceptives and premenopausal bilateral breast cancer, a case-control study. Epidemiol 1992;3:414-9.
15. Bernstein LE, Henderson BE, Hanisch R, Sullivan-Halley J, Ross RK. Physical exercise and reduced risk of breast cancer in young women. J Natl Cancer Inst 1994;86:1403-8.
16. Bernstein LE, Hanisch R, Sullivan-Halley J, Ross RK. Treatment with human chorionic gonadotropin and risk of breast cancer. Cancer Epidemiol Biomarkers & Prev 1995;4:437-40.